Terpene Alkaloids from *Tripterygium wilfordii*

By: Xiao-Dong Wang, Wei Jia, Wen-Yuan Gao, Rong Zhang, Yan-Wen Zhang, Jun Zhang, Yoshihisa Takaishi, and Hong-Quan Duan

Wang, X.-D., Jia, W., Gao, W.-Y., Zhang, R., Zhang, Y.-W., Zhang, J., Takaishi, Y., & Duan, H.-Q. (2005). Terpene alkaloids from *Tripterygium wilfordii*. *Journal of Asian Natural Products Research, 7*, 755-759. doi: 10.1080/1028602042000325618

Abstract:

Two new sesquiterpene alkaloids, 1β-hydroxy-2β,5α,11-triacetoxy-7β-nicotinoyl-8β-benzoyl-dihydroagarofuran (**1**), and 1β,5α,11-triacetoxy-7β-nicotinoyl-8β-benzoyl-dihydroagarofuran (**2**) were isolated from the xylem of *Tripterygium wilfordii*, together with six known compounds. Their structures were elucidated on the basis of spectroscopic studies.

Article:

INTRODUCTION

Plants of the genus *Tripterygium* (Celastraceae) have been used in traditional Chinese medicine for treatment of cancer and as an insecticide for hundreds of years. Over the past several decades, the plants of the genus *Tripterygium*, particularly, their xylem extracts, have widely been used in clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases [1-3]. We have previously reported some anti-HIV agents, triptonines A and B, along with several related compounds from *Tripterygium wilfordii* in our studies on the bioactive metabolites of this genus [4-6]. This paper deals with the isolation and structure determination of two new sesquiterpene alkaloids named wilforsinines A (**1**) and B (**2**), as well as six known compounds (**3**–**8**) from the xylem of *Tripterygium wilfordii*. Compounds **3**–**8** were isolated from the xylem of this plant for the first time.

RESULTS AND DISCUSSION

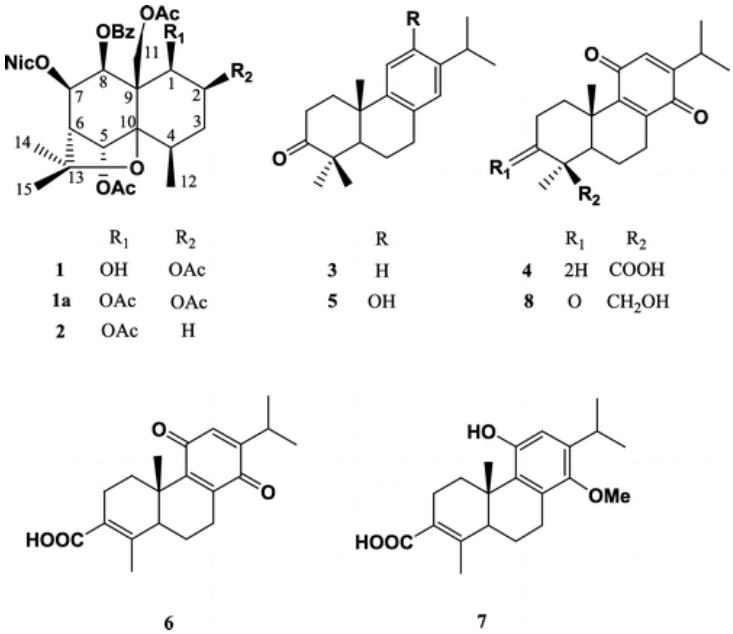
Wilforsinine A (**1**) was obtained as maize crystal, having a molecular formula of $C_{34}H_{39}O_{12}N$ from HREIMS. There was an ester carbonyl band at 1735 cm^{-1} in the IR spectrum, and the UV spectrum showed the presence of an aromatic moiety (225 and 265 nm). The ^{1}H NMR spectral data of **1** revealed the presence of three acetyl methyls (δ_H 2.12, 2.02, and 1.94), a nicotinoyl group [δ_H 9.25 (1H, d, J = 1.6 Hz), 8.81 (1H, br t, J = 3.3 Hz), 8.40 (1H, br d, J = 8.0 Hz), 7.47 (1H, m)], a benzoyl group [δ_H 7.85 (2H, d, J = 7.8 Hz), 7.51 (1H, *t*, J = 7.4 Hz), 7.34 (2H, dd, J = 7.8, 7.4 Hz)], an oxygenated methylene [δ_H 5.26, 4.94 (each 1H, d, J = 12.4 Hz)], as well as five methine protons (δ_H 6.23, 5.96, 5.86, 5.23 and 4.39). The ^{13}C NMR spectral data of **1** revealed the presence of six methyls, one oxygenated methylene, and five oxygenated methine

carbons, in addition to two methines, five ester carbonyl carbons, three quaternary carbons, one nicotinoyl group [δ_C 164.5 (s), 153.6 (d), 151.0 (d), 137.3 (d), 125.9 (s), 123.5 (d)], and one benzoyl group [δ_C 165.2 (s), 133.2 (d), 129.8 (s), 129.5 (d), 128.4 (d)]. From the above information, compound **1** should be a sesquiterpene polyol ester having a dihydroagarofuran skeleton as found in the genus *Tripterygium* [7-9].

The ^{1}H–^{1}H COSY spectrum of **1** revealed two separated spin–spin system (H-1/H-2/H-3/H-4, H-6/H-7/H-8) in the dihydroagarofuran skeleton. The remaining dihydroagarofuran proton signal at δ_H 6.23 (H-5) was correlated with the carbon signals at δ_C 53.2 (C-6), 73.7 (C-7), 50.8 (C-9), 89.8 (C-10) and 81.4 (C-13) in the HMBC spectrum.

From the HMBC spectrum, the proton signal of benzoyl (δ_H 7.85) and the methine proton signal (δ_H 5.96, H-8) were correlated with the carbonyl carbon signal at δ_C 165.2, and the proton signal at δ_H 5.86 (H-7) with the resonance at δ_C 164.5 (nicotinoyl), while the signals at δ_H 5.23 (H-2), 6.23 (H-5) and 4.94 (H-11a) were correlated with the acetyl carbonyl carbons at δ_C 170.6, 169.7, and 169.8, respectively. From above observations, the nicotinoyl and benzoyl groups were assigned at positions C-7 and C-8, and three acetyl groups were assigned at positions C-2, C-5 and C-11, respectively. Acetylation of **1** afforded **1a**, the proton signal at δ_H 4.39 (H-1, in **1**) shifted to the downfield region at δ_H 5.60 in **1a**. Thus, the hydroxyl group was located at position C-1.

In the NOESY spectrum of **1**, the proton signal at δ_H 4.39 (H-1) correlated with the signals at δ_H 5.96 (H-8) and 5.23 (H-2), the proton signal at δ_H 5.96 (H-8) with the signal at δ_H 5.86 (H-7) and 1.64 (H_3-14), and the proton signal at δ_H 5.26 (H-11b) correlated with the signals at δ_H 6.23 (H-5) and 1.23 (H_3-12). Thus, the relative stereochemistry of the ester and hydroxyl groups were elucidated as having the 1β, 2β, 5α, 7β and 8β configurations. The ^{1}H and ^{13}C NMR assignments were obtained by 2D NMR spectra including NOESY. Therefore, the structure of wilforsinine A (**1**) was determined as shown in figure 1.



	R_1	R_2
1	OH	OAc
1a	OAc	OAc
2	OAc	H

3	R	H
5		OH

	R_1	R_2
4	2H	COOH
8	O	CH_2OH

6 **7**

Figure 1: Structures of compounds **1**–**8**.

Wilforsinine B (**2**), $C_{34}H_{39}O_{11}N$, revealed signals for three acetyl groups (δ_H 2.17, 1.97, and 1.49), a benzoyl group [δ_H 7.93 (2H, d, *J* = 7.1 Hz), 7.58 (1H, t, *J* = 7.4 Hz), 7.43 (2H, dd, *J* = 7.4, 7.1 Hz)], and a nicotinoyl group [δ_H 9.14 (1H, br s), 8.75 (1H, m), 8.31 (1H, br d, *J* = 8.0 Hz), 7.55 (1H, m)], as well as four methine protons [δ_H 6.82, 5.84, 5.81, 5.47] in its ^{1}H NMR spectrum. The ^{13}C NMR spectral data were similar to those of **1**, except for the C-1, -2 and -3 carbon signals (table 1). Compound **2** was also a dihydroagarofuran polyol ester and was presumed to be 1-acetyl-2-deacetoxy of **1**. In the HMBC spectrum of **2**, the proton signals at δ_H 8.31 (nicotinoyl) and 5.84 (H-7) correlated with the carbonyl carbon signal at δ_C 165.9, and the signals at δ_H 7.93 (benzoyl group) and 5.81 (H-8) with the carbon signal at δ_C 166.4, while the signals at δ_H 5.47 (H-1), 6.82 (H-5) and 4.83 (H-11a) correlated with the acetyl carbon signals at δ_C 171.8, 171.7 and 172.2, respectively. Thus, the nicotinoyl and benzoyl groups were assigned at positions C-7 and C-8, and three acetyl groups were located at positions C-1, C-5 and C-11. In the NOESY spectrum, the proton signal at δ_H 4.83 (H-11a) correlated with the proton signals at 6.82 (H-5) and 1.07 (H_3-12), and the proton signal at δ_H 5.81 (H-8) with the signals at δ_H 5.47 (H-1) and 1.63 (H_3-14), while the signal at δ_H 5.84 (H-7) correlated with the signal at δ_H 1.63 (H_3-14). Therefore, the relative configurations of ester groups of **2** were determined as 1β, 5α, 7β and 8β (figure 1).

Table 1: ^{1}H NMR and ^{13}C NMR spectral data of **1** and **2**.

	1 (CDCl$_3$)		***2 (CD$_3$OD)***	
No.	***^{13}C***	***^{1}H***	***^{13}C***	***^{1}H***
1	76.6	4.39 (m)	80.7	5.47 (m)
2	73.6	5.23 (m)	24.3	1.83, 1.68 (m)
3	33.2	2.30, 1.90 (m)	27.5	2.24, 1.55 (m)
4	31.2	2.33 (m)	34.9	2.32 (m)
5	74.7	6.23 (s)	76.1	6.82 (s)
6	53.2	2.70 (d, 4.1)	54.6	2.72 (d, 3.8)
7	73.7	5.86 (dd, 5.6, 4.1)	73.2	5.84 (dd, 5.9, 3.8)
8	72.2	5.96 (d, 5.6)	74.4	5.81 (d, 5.9)
9	50.8	–	52.4	–
10	89.8	–	92.2	–
11	64.0	5.26, 4.94 (d, 12.4)	62.0	4.83, 4.72 (d, 13.3)
12	18.1	1.21 (d, 7.7)	15.6	1.07 (d, 7.5)
13	81.4	–	82.5	–
14	24.5	1.64 (s)	24.8	1.63 (s)
15	30.4	1.46 (s)	30.8	1.52 (s)

The known compounds were identified by spectral comparison with 8,11,13-abietatriene-3-one (**3**) [10], triptoquinone F (**4**) [11], hinokione (**5**) [12], triptoquinone A (**6**) [11], triptobenzene H (**7**) [13] and triptoquinone B (**8**) [11], respectively.

EXPERIMENTAL

General experimental procedures

NMR experiments were run on a Bruker AVANCE 300 instrument. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) both had teramethylsilane as an internal standard. MS data were obtained on a JEOL JMS-SX102A instrument. Column chromatography was performed on silica-gel (Qingdao Haiyang Chemical Co. Ltd) and Sephadex LH-20 (Amersham Pharmacia Biotech). HPLC was a JASCO Gulliver Series with PU-1580 (pump), RI-1530 and UV-1575 (detector). Preparative HPLC column was used as follows: ODS (YMC-Pack ODS-A, SH-343-5), GPC (Shodex, Asahipak GS-310, 20G, MeOH), Si-HPLC$_1$ (Hibar RT 250-25, Lichrosorb, Si60 7 μm), and Si-HPLC$_2$ (YMC-pack SIL-06, SH-043-5-06). IR spectra were recorded on a 1710 Infrared Fourier Transform spectrometer (Perkin-Elmer). UV spectra were obtained on a UVIKON$_{XS}$ recording

spectrometer (Bio-Tek). Optical rotation was measured with a MC 241 digital polarimeter (Perkin-Elmer).

Plant material

The xylem rhizomes of *Tripterygium wilfordii* were purchased from Yueyang, Hunan province, and were identified by Professor Wen-Yuan Gao, Department of Pharmacognosy and Natural Medicines, Tianjin University. A voucher specimen (D20021018) is deposited at the College of Pharmaceuticals and Biotechnology, Tianjin University, China.

Extraction and isolation

The xylem rhizomes (10 kg) of *T. wilfordii* were refluxed three times with 95% EtOH (15 l each) for 2 h. The extract was concentrated under reduced pressure to give a residue (390 g) which was partitioned between chloroform and H_2O. The $CHCl_3$ layer was concentrated to a residue of 112 g. Chromatographic separation was performed with a silica gel column and solvents of increasing polarity as mobile phase [petroleum ether/EtOAc (8:1, 5:1, 3:1, 1:1, 1:2, 1:4), EtOAc, EtOAc/MeOH (19:1, 9:1, 4:1), MeOH] to give 16 frs. Fraction 10 (2 g) was chromatographed on Sephadex LH-20 (MeOH) to give 3 frs. (fr. 10.1–10.3). Fr. 10.1 (840 mg) was separated by HPLC (ODS, MeOH/H_2O 8:2) to give 12 frs. (fr. 10.1.1.1–10.1.1.12). Fr. 10.1.1.5 (80 mg) and Fr. 10.1.1.9 (26 mg) were separated respectively by HPLC (ODS, MeOH/H_2O 7:3) to give **1** (6.5 mg) and **2** (8.5 mg). Fraction 7 (2.4 g) was chromatographed on a silica column [$CHCl_3$/MeOH (97:3, 9:1)] to give 10 frs. (fr.7.1–7.10). Fr. 7.7 (85 mg) was separated by HPLC (GPC, MeOH) to give **3** (6.5 mg). Fraction 7.3 (210 mg) was separated by Sephadex LH-20 (MeOH) to give **4** (21 mg). Fraction 6 (2.2 g) was chromatographed on Sephadex LH-20 (MeOH), then separated by Si-$HPLC_1$ ($CHCl_3$/MeOH 97:3) to give **5** (5.0 mg). Fraction 11 (8.5 g) was chromatographed on a silica column to give frs. 11.1–11.8. Fraction 11.5 (800 mg) was separated by GPC (MeOH), then by Si-$HPLC_2$ (hexane/EtOAc 3:1) to give **6** (8.0 mg) and **7** (90 mg). Fraction 13 (5.2 g) was chromatographed with middle pressure silica gel column with $CHCl_3$/MeOH (98:2, 95:5, 9:1) to give 10 frs. (fr. 13.1–13.10). Fraction 13.6 (245 mg) was chromatographed using LH-20 (MeOH), then by Si-$HPLC_2$ (hexane/EtOAc 5:2) to give **8** (50 mg).

Wilforsinine A (**1**) was isolated as a maize crystal. $[\alpha]_D^{25}$ − 10.5 (c 0.1, MeOH). UV (MeOH) λ_{max} (log ϵ): 225 (4.21), 265 (3.57) nm. IR (KBr) ν_{max} cm^{-1}: 3438, 2920, 2851, 1735, 1593, 1371, 1292, 1225, 1106, 1042, 743, 713. ^{1}H-NMR ($CDCl_3$), see table 1; δ 2.02 (2-OAc); 2.12 (5-OAc); 1.94 (11-OAc); 7.85 (2H, d, *J* = 7.8 Hz), 7.51 (1H, t, *J* = 7.4 Hz), 7.34 (2H, dd, *J* = 7.8, 7.4 Hz), (8-OBz); 9.25 (1H, d, *J* = 1.6 Hz), 8.81 (1H, br t, *J* = 3.3 Hz), 8.40 (1H, br d, *J* = 8.0 Hz), 7.47 (1H, m), (7-ONic). ^{13}C-NMR ($CDCl_3$), see table 1; δ 21.3, 170.6 (2-OAc); 21.2, 169.7 (5-OAc); 21.2, 169.8 (11-OAc); 165.2, 129.5, 128.4, 133.2 (8-OBz); 164.5, 125.9, 137.3, 123.5, 153.6, 151.0 (7-ONic). EI-MS: *m*/*z* 653[M]$^+$(3), 611 (5), 593 (7), 318 (4), 149 (15), 124 (36), 105 (100), 57 (24). HR-EIMS *m*/*z* 653.2457 (calcd for $C_{34}H_{39}O_{12}N$, 653.2472).

Compound **1** was subjected to acetylation with Ac_2O-pyridine for 4 h at room temperature to give **1a**. ^{1}H-NMR ($CDCl_3$), δ 5.60 (1H, d, *J* = 3.2 Hz, H-1), 5.41 (1H, m, H-2), 2.50 (m, H-3), 1.95 (m, H-3), 2.35 (m, H-4), 6.85 (s, H-5), 2.66 (1H, d, *J* = 3.7 Hz, H-6), 5.79 (1H, m), 5.76 (1H, m), 5.36 (1H, d, *J* = 12.5 Hz, H-11), 4.63 (1H, d, *J* = 13.5 Hz, H-11), 1.16 (1H, d, *J* = 7.7 Hz), 1.53 (3H, s), 1.48 (3H, s), 1.62 (3H, s), 2.08 (3H, s), 2.15 (3H, s), 2.00 (3H, s), 7.90

(2H, d, *J* = 7.2 Hz), 7.70 (1H, m), 7.38 (2H, br t, *J* = 7.2 Hz), (8-OBz); 9.22 (1H, *s*), 8.78 (1H, br t, *J* = 7.4 Hz), 8.27 (1H, br d, *J* = 7.4 Hz), 7.53 (1H, *m*) (7-ONic).

Wilforsinine B (**2**) was isolated as a colourless crystal. $[\alpha]_D^{25}$ − 34.1 (c 0.1, MeOH). UV (MeOH) λ_{max} (log ϵ) δ 225 (4.23), 265 (3.61) nm. IR (KBr) ν_{max} cm^{-1}: 3448, 2927, 1738, 1592, 1452, 1371, 1231, 1097, 1025, 743, 713. ^{1}H-NMR (CD_3OD), see table 1; δ 1.49 (1-OAc); 2.17 (5-OAc); 1.97 (11-OAc); 7.93 (2H, d, *J* = 7.1 Hz), 7.58 (1H, t, *J* = 7.4 Hz), 7.43 (2H, dd, *J* = 7.4, 7.1 Hz), (8-OBz); 9.14 (1H, br s), 8.75 (1H, m), 8.31 (1H, br d, *J* = 8.0 Hz), 7.55 (1H, m) (7-ONic). ^{13}C-NMR (CD_3OD), see table 1; δ 21.2, 171.7 (1-OAc); 21.6, 172.2 (5-OAc); 21.4, 171.8 (11-OAc); 166.4, 131.5, 130.8, 129.9, 134.9 (8-OBz); 165.9, 128.0, 139.2, 125.4, 154.5, 151.5 (7-ONic). EI-MS: *m*/*z* 637[M]$^+$(27), 595 (78), 124 (43), 106 (44), 105 (100), 77 (15). HR-EIMS *m*/*z* 637.2556 (calcd for $C_{34}H_{39}O_{11}N$, 637.2523).

REFERENCES

[1] S.Z. Qian. *Contraception*, **36**, 335 (1987).
[2] S.A. Matlin, A. Belenguer, V.E. Stacey, S.Z. Qian, Y. Xu, J.W. Zhang, J.K.M. Sanders, S.R. Amor, C.M. Pearce. *Contraception*, **47**, 387 (1993).
[3] S.Z. Qian, Y. Xu, J.W. Zhang. *Contraception*, **51**, 121 (1995).
[4] H.Q. Duan, T. Yoshihisa, T. Hiroshi, M. Yasukazu, T. Takao, Y.F. Jia, D. Li. *J. Nat. Prod.*, **64**, 582 (2001).
[5] H.Q. Duan, T. Yoshihisa, I. Yasuhiro, Y.F. Jia, D. Li, K.H. Lee. J. *Nat. Prod.*, **63**, 357 (2000).
[6] H.Q. Duan, T. Yoshihisa, Y.F. Jia, D. Li. *Chem. Pharm. Bull.*, **47**, 1664 (1999).
[7] T. Yoshihisa, K. Tokura, S. Tamai, K. Ujita, K. Nakano, T. Tomimatsu. *Phytochemistry*, **30**, 1567 (1991).
[8] T. Yoshihisa, S. Tamai, K. Nakano, T. Tomimatsu. *Phytochemistry*, **30**, 3027 (1991).
[9] H.Q. Duan, T. Yoshihisa, M. Hiroshi, O. Yasukazu, T. Takao, Y.F. Jia, D. Li. *Phytochemistry*, **53**, 805 (2000).
[10] G. Topca, A. Vlubelen. *Phytochemistry*, **29**, 2346 (1990).
[11] S. Kozo, N. Kimiko, K. Kazuyoshi, T. Yoshihisa. *Phytochemistry*, **35**, 731 (1994).
[12] M. Takashi, U. Shuj, K. Hiroyuk, M. Masanori. *Bull. Chem. Soc. Jpn*, **54**, 581 (1981).
[13] K.H. Li, H.Q. Duan, K. Kazuyoshi, T. Yoshihisa. *Phytochemistry*, **35**, 791 (1997).